Duration and Spread of an Entomopathogenic Fungus, *Beauveria bassiana* (Deuteromycota: Hyphomycetes), Used to Treat Varroa Mites (Acari: Varroidae) in Honey Bee (Hymenoptera: Apidae) Hives

W. G. MEIKLE,[1,2] G. MERCADIER,[1] N. HOLST,[3] C. NANSEN,[4] AND V. GIROD[5]

J. Econ. Entomol. 100(1): 1–10 (2007)

ABSTRACT A strain of the fungus *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolated from varroa mites, *Varroa destructor* Anderson & Trueman (Acari: Varroidae), was used to treat honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), against varroa mites in southern France. Fungal treatment caused a significant increase in the percentage of infected varroa mites compared with control treatments in two field experiments. In the first experiment, hives were treated with a formulation containing 0.37 g of *B. bassiana* conidia per hive and in the second experiment with a dose of 1.0 g of conidia per hive. The percentage of infected varroa mites also increased in the nontreated (control) hives, suggesting a movement of conidia, probably via bee drift, among the hives. Mite fall was significantly higher among treated hives compared with control hives on the sixth and eighth days after treatment in the first experiment. These days correspond to previously published data on the median survivorship of mites exposed to that fungal solate. The interaction of treatment and date was significant in the second experiment with respect to mite fall. Increases in colony-forming unit (cfu) density per bee were observed in all treatments but were significantly higher among bees from treated hives than control hives for at least a week after treatment. The relationship between cfu density per bee and proportion infected was modeled using a sigmoid curve. High levels of infection (>80%) were observed for cfu density per bee as low as 5×10^2 per bee, but the cfu density in hives treated with 0.37 g generally dropped below this level less than a week after treatment.

KEY WORDS *Apis mellifera*, *Varroa destructor*, *Beauveria bassiana*, hive treatment

Varroa mites, *Varroa destructor* Anderson & Trueman (Acari: Varroidae) are an important pest of honey bees (Chandler et al. 2001, Rinderer et al. 2001). Originally from eastern Asia, *V. destructor* was found in continental Europe, northern Africa and South America by 1975 (De Jong et al. 1982) and was first detected in the United States in 1987 (Chandler et al. 2001). Infested colonies often die within 2 yr. The mites do not cause massive acute mortality, but they weaken larvae and adults by feeding on hemolymph, transmitting diseases, and inducing deformities (Chandler et al. 2001, Martin 2001). The impact of varroa mites on colonies of honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), in many regions has been profound; feral populations of *A. mellifera*, once common, have been almost eliminated by the mites (Rinderer et al. 2001). The loss of wild colonies of *A. mellifera* has been felt most by farmers who depend on the bees for the pollination of fruit and field crops.

Chemical control of varroa mites has some drawbacks. Apart from concerns of residues in wax and honey, mite populations resistant to the most common chemical pesticides, fluvalinate and coumaphos, have been observed (Elzen et al. 1998, Milani 1999, Elzen and Westervelt 2002). Biopesticides, and in particular entomopathogenic fungi, can be used as alternatives to chemical insecticides in some agricultural systems. For beekeepers, preparations of *Bacillus thuringensis* Berliner are registered for the use against wax moth, *Galleria mellonella* L., larvae in Europe. Several species of entomopathogenic fungi, including *Beauveria bassiana* (Balsamo) Viullemin (Deuteromycota: Hyphomycetes), have been found to infect varroa mites in the laboratory (Chandler et al. 2001, Kanga et al. 2002, Shaw et al. 2002, Davidson et al. 2003, Meikle et al. 2006b), and in the only published studies directly comparable to this study, both *Hirsutella thompsonii* Fisher and *Metarhizium anisopliae* (Metschnikoff) have been shown to affect mite densities in honey bee colonies (Kanga et al. 2002, 2003, 2005).

[1] European Biological Control Laboratory, USDA–ARS, Campus International de Baillarguet, CS 90013 Montferrier sur Lez, 34988 St. Gely du Fesc, France.
[2] Corresponding author, e-mail: wmeikle@ars-ebcl.org.
[3] Danish Institute of Agricultural Sciences, Flakkebjerg, 4200 Slagelse, Denmark.
[4] Exosect Ltd, Leyland Farm, Colden Common, Winchester, SO16 7NP, United Kingdom.
[5] ADAPRO LR-CRALR, Maison des Agriculteurs, Mas de Saporta CS 30012, 34875 Lattes, France.

Meikle et al. (2006b) found *B. bassiana* on varroa mites collected from beehives in southern France and tested two isolates in laboratory bioassays on varroa mites. The objective of the bioassays was to compare the survivorship of mites treated with spores in a spray tower with controls (mites treated with a blank solution). One isolate, selected for the field experiments described here, was tested twice, and the median survivorships of mites treated with that isolate were 5 and 8 d, whereas survivorship in the control treatments was 23.5 and 26 d, respectively. Meikle et al. (2006b) also found that all bee pupae kept with treated mites eventually sporulated, whereas none kept with control mites sporulated. Although some additional infection of bee pupae may be expected, given that bee pupae in laboratory bioassays are kept in a stressful environment different from a sealed cell in a beehive, these data do suggest that the populations of adult and larval bees in the beehives treated with this fungus should be monitored to exclude the possibility of an epizootic in the hive.

Two criteria for evaluating a fungal biopesticide are 1) treatment-induced mortality of the target pest and 2) the change in the percentage of infected pests before and after treatment. Other important variables are the duration of the treatment effect, measured as the length of time the percentage of infected pests exceeds background levels, and the spread of the treatment among untreated pest populations, in this case among untreated hives. The objectives of this study were to 1) apply formulated conidia of a strain of *B. bassiana* to beehives, with control treatments in the form of blank formulation and no treatment; 2) observe mite fall and the percentage of infected mites before and after treatment; and 3) measure the density of colony-forming units (cfu) over time and relate that to percentage of infected mites.

Materials and Methods

Preparation of Formulation. Cultures of *B. bassiana* isolate Bb05002, isolated from varroa mites in southern France (Meikle et al. 2006b) were grown on Sabouraud dextrose agar with yeast (SDAY) (Goettel and Inglis 1997) for 15 d. Conidia were harvested by scraping the tops of the cultures onto glass petri dishes with a metal spatula, and the petri dishes placed in a crystallizing dish containing silica gel for drying. The viability of the conidia was assessed by plating a suspension sample onto SDAY, incubating the plates at 23°C for 24 h, and examining 200 conidia for germ tubes under a light microscope. Conidia were formulated as follows: Bb05002 conidia were mixed with Entostat powder, a refined and electrostatically chargeable carnauba wax powder (Exosect, Winchester, United Kingdom) and a small amount of hydrated silica (Hi-Sil-233, Pittsburgh Plate Glass, Pittsburgh, PA) by using a food processor (Valentin Mini Chopper, SEB, Dijon, France). The exact proportions for each experiment are given for each experiment below. The density of viable conidia per gram of formulation was determined by plating onto potato-dextrose agar three subsamples of the formulation diluted in distilled water and Tween 80 (Merck, Munich, Germany), and by counting the number of colonies 96 h after plating.

First Field Experiment. The first field experiment was conducted at the European Biological Control Laboratory (EBCL), Montferrier sur Lez, France. Eight bee colonies, four established in April 2004 and four established in May 2005, were maintained in painted, 10-frame, wooden Dadant brood boxes (56-liter capacity) (Ickowicz, Bollène, France). The hives were covered with telescoping lids with a weight placed on top to stabilize the hive in wind. The hives were arranged in two groups of four hives each, 4 m apart. Within each group hives were kept ≈50 cm apart. Permanent water sources existed <1 km away. To monitor changes in hive weight during the course of the experiment, four of the hives, two in each hive group, were each placed on top of two short wooden planks resting on stainless steel electronic balances (TEKFA model B-2418, Galten, Denmark) (Meikle et al. 2006a). The balances had a 100-kg maximum capacity, a precision of 10 g, and an operating temperature range of −30 to 70°C. The balances were linked to 12-bit dataloggers (Hobo U-12 External Channel datalogger, Onset Computer Corporation, Bourne, MA) and powered by a solar panel (BP Solar model 1230, Mimeure, France). The weighing system had an overall precision of ≈30 g. Every 2 wk, starting 3 wk before treatment, each of the hives on a balance was opened, and the super and each brood box frame was shaken (to remove bees) and weighed separately with a portable electronic balance (Kern & Sohn model 12K 1N, Balingen, Germany). Adult bee mass was calculated by subtracting the combined weight of the frames (containing brood, honey, and pollen) and noncolony components from the total hive weight before it was opened. Digital photographs were taken of each side of each frame on each sampling occasion by using a 3.3-megapixel Coolpix 990 camera (Nikon, Tokyo, Japan), and the area of sealed brood per photograph was estimated using ArcView 3.0 (Environmental Systems Research Institute, Redlands, CA).

On 14 September 2005, varroa sticky boards (Mann Lake Ltd., Hackensack, MN) were placed under all eight hives in the apiary. On 20 September, the sticky boards were collected from beneath each hive and replaced. Two hives on balances were selected for treatment, one hive established in 2004 and the other hive in 2005. For each treated hive, a plastic laboratory wash bottle (Nalge Nunc International, Rochester, NY) was filled with formulation containing 0.38 g of Bb05002 conidia + 10.0 g of Entostat powder + 0.05 g of HiSil, and the formulation was blown between all the frames in the brood box by squeezing the wash bottle. The other two hives on balances, one hive established in 2004 and the other hive in 2005, were treated in a similar manner with formulation containing only 10.0 g of Entostat powder and 0.05 g of silica. The remaining hives in the apiary were not treated. A weather station (HOBO micro station, Onset Com-

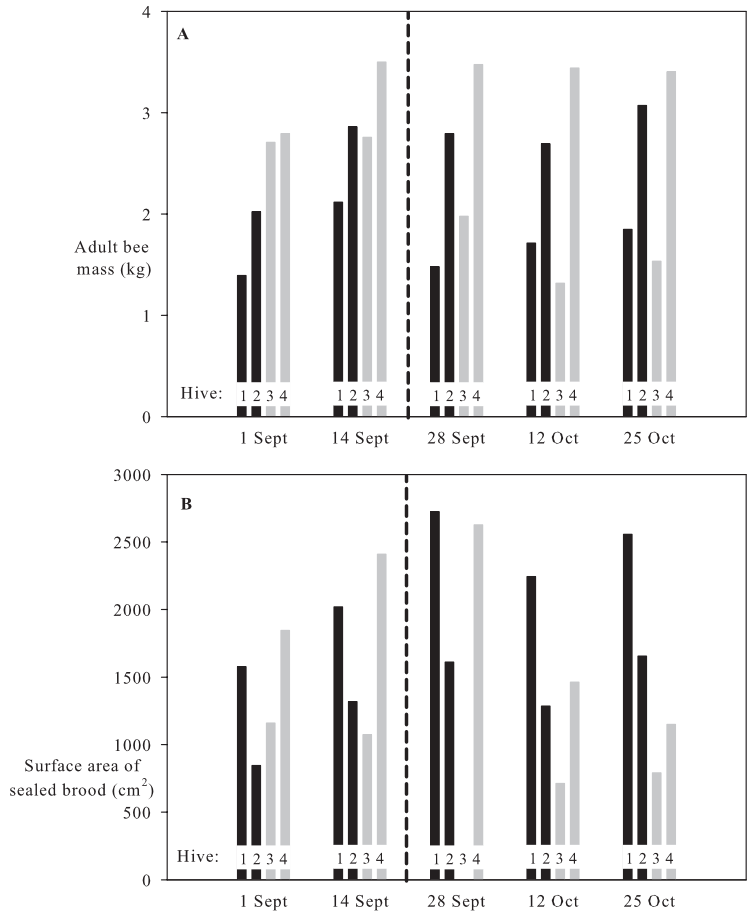

Fig. 1. Measurements of hive health for an experiment conducted at EBCL near Prades le Lez, France. (A) Estimated weights of adult bees and (B) surface area of sealed brood, in square centimeters, for four hives treated with either Entostat powder + conidia (black columns) or Entostat powder alone (gray columns). Vertical dashed lines show treatment date.

puter Corporation) was used to monitor ambient temperature, relative humidity, and rainfall.

Sticky boards were removed and replaced 2 d after treatment to reduce contamination due to formulation on the boards. For the next 17 d, each hive had two sets of boards, with a given board being used on alternate days, and a given board was always used for the same hive. All the mites on each board were counted, removed from the board, and plated on water agar (6.0 g/liter) with chloramphenicol (0.4 g/liter). Thereafter, sticky boards were replaced twice a week, all the mites counted, and 40-mite samples were taken from each board and plated on agar. If a board had 40 mites or fewer, all mites were plated. After plating, all mite samples were incubated at 23°C and examined for sporulation after 15 d.

The day before treatment, and 1, 3, 8, 16, and 24 d after treatment, samples of ≈15 bees were collected from within each hive, placed in small plastic bags and immediately placed in a freezer for 3 wk. Three subsamples of five bees each were removed from each bag and each subsample was placed in a 50-ml plastic centrifuge tube and vortexed for 3 min in 10 ml of a 0.1% sterile aqueous solution of Tween 80. Aliquots of 20 and 100 μl of the resulting suspension from each subsample were spread onto each of three petri dishes containing SDAY with chloramphenicol (0.4 g/liter); thus, nine plates for each sampling occasion. The dishes were incubated for 14 d at 23°C, and the number of *B. bassiana* cfu were counted in the plates with 20 μl of solution; when cfu densities became low, cfu were counted on the 100-μl plates.

Second Field Experiment. The second experiment was conducted near Lattes, ≈15 km from EBCL. Eleven hives, each containing ≈2–4 kg of adult bees and at least 6 mo old, were selected in an apiary containing ≈50 hives. As described above, all hives were wooden, 10-frame Dadant hives. Experimental hives were separated by one or more hives not included in the experiment. Sticky boards were placed under all hives on 3 October 2005. The boards were removed before treatment on 10 October 2005, mites were counted and sampled, and new boards were placed under the hives just after treatment. Four ran-

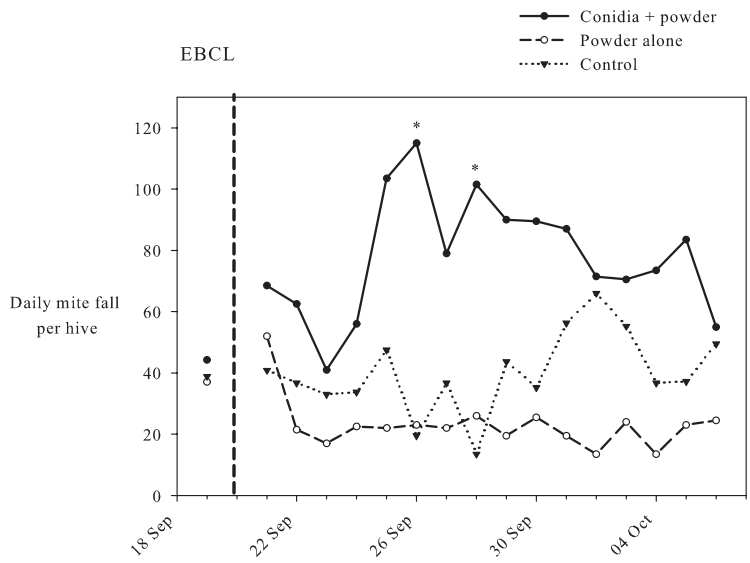

Fig. 2. Average daily mite fall per hive at EBCL near Prades le Lez, France, for bee hives treated on 20 September 2005 with *B. bassiana* conidia and electrostatic powder (solid line), electrostatic powder only (dashed line), or nothing (control; dotted line). Points with an asterisk indicate a significant difference between hives treated with conidia and control hives ($\alpha = 0.05$) on that day. Vertical dashed line shows treatment date.

domly selected hives were treated, using the technique described above, with formulation containing 1.0 g of Bb05002 conidia + 10 g of Entostat powder + 0.05 g of HiSil. Three hives were treated with 10 g of Entostat powder + 0.05 g of HiSil, and four hives were chosen as untreated controls. Sticky boards were replaced every 3–4 d after treatment for 3 wk, and weekly thereafter for 2 wk. All mites were counted and 40-mite samples from each board were plated as described for the first experiment. Hives were not weighed.

Statistical Analysis. Data were analyzed using SAS and JMP (SAS Institute, Inc., Cary NC) software. Multiple regression analyses ($\alpha = 0.05$) were analyzed with a linear mixed model using PROC MIXED of SAS (Littell et al. 1996) with either daily mite fall (square root transformed), proportion infected mites (arcsine-square root-transformed) or cfu density per bee [log(x + 1) transformed] as response variable and with three fixed effects: treatment, date, and their interaction. Hive number was incorporated in the analysis as a random effect. The degrees of freedom were calculated using the Satterthwaite method. For each variable, the covariance matrix of the response variable was inspected for patterns and residual plots were assessed visually to check variance homogeneity. Insignificant main effects were excluded from the model but if the interaction was significant both main factors were retained. Because mite fall due to powder alone could be expected (Macedo et al. 2002), and because excess formulation on sticky boards immediately after treatment may cause spurious infection data, the first sample after treatment was excluded from each of the analyses of mite fall and proportion infection.

Results

Conidia density was 7.95×10^9 conidia per g formulation for the first field experiment at EBCL and 1.26×10^{10} conidia per g formulation for the second field experiment in Lattes. Viability of the conidia in both experiments was ≈91%. No patterns, such as autocorrelation through time, were detected in the covariance matrix, and no problems were observed in the residual plots of any analysis for either experiment. In both experiments, a low percentage of mites was naturally infected based on samples taken 7 d before treatment (5.0 ± 4.0 SD % at EBCL and 1.1 ± 1.9 SD % at Lattes).

First Field Experiment. Adult bee masses were never significantly different between the powder only treatment and the conidia + powder treatment (*t*-test within sampling occasion, $\alpha = 0.05$) (Fig. 1). One hive treated with powder only lost its queen in the first half of September; although the queen was replaced before the start of the experiment, a visual inspection of the frame photos for that hive (hive 3) showed an absence of brood at the first sampling after treatment. Because of the importance of brood to varroa dynamics, mite fall data for hive three were excluded from statistical analyses, although data on percentage infected mites for hive three were retained. Only one other hive (hive 4) was treated only with powder, so the powder treatment was removed from the mite fall analyses for the EBCL experiment.

The average daily mite fall per hive was calculated for the first 17 d after treatment (Fig. 2). Mixed model analysis of mite fall showed that although treatment was not significant ($F_{1,\ 4} = 1.60$; $P = 0.274$), both date ($F_{13,\ 52} = 2.45$; $P = 0.0113$) and their interaction

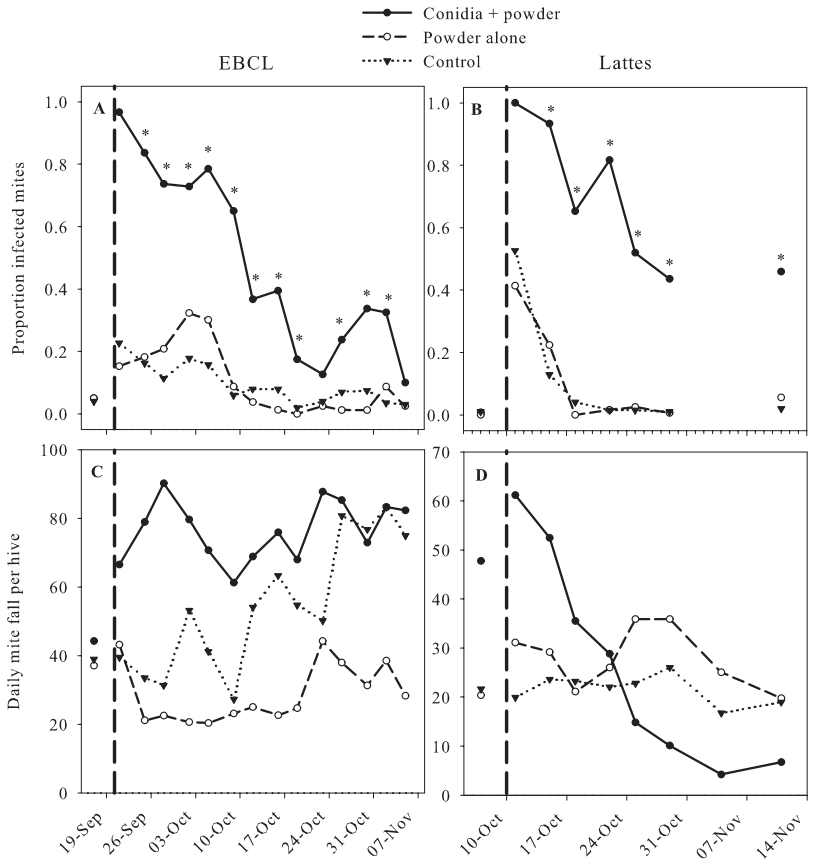

Fig. 3. Average proportion infected mites and average daily mite fall per hive over time for hives treated with *B. bassiana* conidia and electrostatic powder (solid line), electrostatic powder only (dashed line), or nothing (control; dotted line). (A) Percentage infected mites at EBCL site; (B) percentage infected mites at Lattes site; (C) mite fall at EBCL site; and (D) mite fall at Lattes site. Within each sampling occasion, points with an asterisk (*) on top indicate that the treated colonies were significantly different (Tukey's honestly significant different (HSD) with $P = 0.05$) from both the powder only and control treatments. Vertical dashed lines show treatment dates.

($F_{13, 52} = 4.38$; $P < 0.0001$) were significant, indicating that mite fall did exhibit significant change over time and that the relationship between the treated and control hives varied over time. Post hoc contrasts showed that hives treated with conidia had significantly higher mite fall than control hives on 26 ($F_{1, 5} = 8.13$; $P = 0.0333$) and 28 September ($F_{1, 5} = 7.33$; $P = 0.0398$), that is, 6 and 8 d after treatment, which was when maximum treatment effect was expected based on laboratory bioassays (Meikle et al. 2006b).

The daily data up to 6 October were pooled to generate semiweekly data and combined with later data collected twice per week (Fig. 3). A mixed model analysis of this semiweekly data was conducted for percentage infection and mite fall. In the percentage of infection analysis, both treatment and date were significant ($F_{2, 5} = 60.78$; $P = 0.0003$ for treatment; $F_{12, 60} = 11.96$; $P < 0.0001$ for date), but interaction was not significant ($F_{24, 60} = 3.16$; $P = 0.089$), indicating that treatment caused a significant increase in the incidence of infection and that the percentages varied over time. The nonsignificant interaction means that the treatment effects on the transformed scale were approximately constant. After removing the interaction term and reanalyzing the data by using treatment contrasts with the Bonferroni method (Marascuilo and Levin 1983), the conidia + powder treatment was shown significantly different from both the powder only treatment ($t_7 = 6.06$, $P = 0.0015$) and from the control ($t_7 = 6.61$, $P = 0.0009$). After treatment, infected mites were found in substantial numbers also in untreated hives in both experiments. In the mite fall analysis, none of the factors was significant ($F_{1, 4} = 0.22$, $P = 0.665$ for treatment; $F_{12, 48} = 1.95$; $P = 0.052$ for date; $F_{12, 48} = 1.02$, $P = 0.444$ for interaction). After removing the interaction term and reanalyzing the data, date was significant ($F_{12, 60} = 2.65$; $P = 0.0065$), but treatment was not significant ($F_{1, 4} = 0.22$, $P = 0.665$).

Analysis of the cfu density per bee showed that treatment ($F_{2, 5} = 43.68$, $P = 0.0007$), date ($F_{4, 20} = 30.40$, $P < 0.0001$), and their interaction ($F_{8, 20} = 20.24$; $P < 0.0001$) were significant. Low numbers of *B. bassiana* cfu, averaging ≈1 cfu per bee, were found the

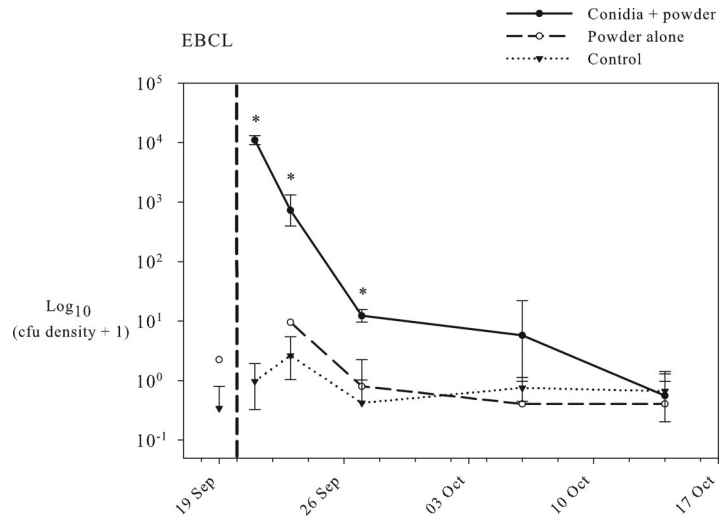

Fig. 4. Average number of *B. bassiana* cfu per adult bee by treatment over time at EBCL site. Bars show back-transformed SE intervals. Within each sampling occasion, points with an "*" on top indicate that the treated colonies were significantly different (Tukey's HSD with $P = 0.05$) from both the powder only and control treatments. Vertical dashed line shows treatment date.

day before treatment (Fig. 4). The day after treatment the number of cfu peaked at an average of $\approx 1.1 \times 10^4$ cfu per bee in the treated hives, and declined rapidly thereafter. Elevated cfu levels also were observed in bees from control hives and those treated only with powder. However, the average levels never exceeded 10 cfu per bee in the powder-only treatment and 3 cfu per bee in the control. After 24 d, cfu densities on bees were very similar among treatments, and they were similar to the pretreatment level. The response of percentage infected mites to cfu density on bees was sigmoid (Fig. 5).

Second Field Experiment. One treated hive was found to have either swarmed or died during the course of the Lattes experiment; data for that hive were not included in the analyses. Percentage of infection data for 1 week, 1–7 November, was lost due to contamination. A mixed model analysis of the percentage infection data showed that all the factors, treatment ($F_{2,\ 7} = 26.31$; $P = 0.0006$), date ($F_{5,\ 35} = 12.06$; $P < 0.0001$), and their interaction ($F_{10,\ 35} = 2.24$; $P = 0.0384$), were significant, and a significantly higher percentage of mites from hives treated with conidia were infected compared with either mites from the

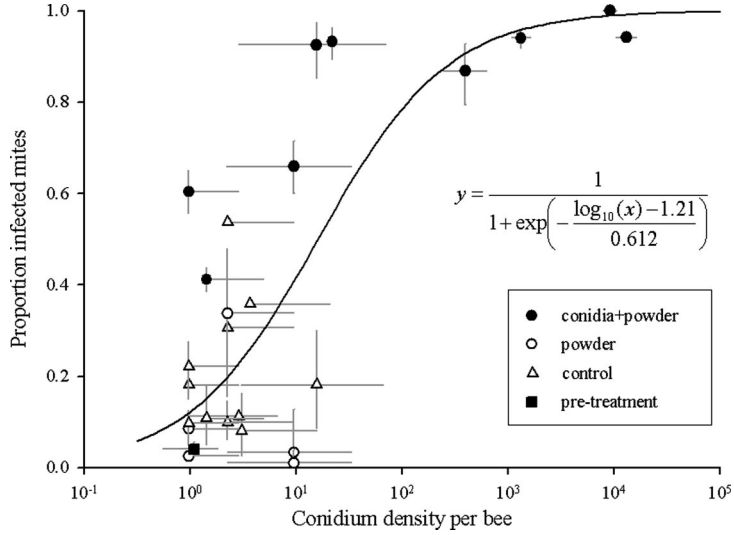

Fig. 5. Proportion infected mites in response to *B. bassiana* cfu density per bee. Each observation is from a single hive showing cfu density from one sampling date and daily mite samples averaged around that date; no mite samples occurs twice. Eighteen observations with zero cfu density are not shown. They had an average ± SD proportion of infected mites of 0.16 ± 0.13. Filled circles, conidia + powder treatment; empty circles, powder alone treatment; and triangles, control.

powder only treatment or mites from the controls on all dates. Analysis of mite fall showed that date ($F_{7, 49} = 6.81$; $P < 0.0001$) and the interaction of treatment and date ($F_{14, 49} = 4.61$; $P < 0.0001$) were significant effects, but treatment was not ($F_{2, 7} = 0.04$; $P < 0.961$). Post hoc analysis did not identify any dates when treatment had a significant effect.

Discussion

This work is the first published account of the application of a hyphomycete fungus obtained from varroa mites in beehives and used against varroa mites in beehives. In a field experiment at EBCL, daily mite fall peaked in the treated hives between 5 and 8 d after treatment, or about the time a peak was anticipated based on bioassay results at 30°C and 80% RH (Meikle et al. 2006b). Treatment caused widespread infection of mites in the treated hives in both experiments, with some infection in untreated hives as well, and the effect lasted over a month after treatment. A small percentage of varroa collected from the hives before treatment also was found to be infected with *B. bassiana,* suggesting that, at least in southern France, these fungi play a role in bee and mite ecology.

A small (not significant) spike in mite fall was observed within 24 h of treatment in the powder alone and conidia + powder treatments in the first experiment. Figure 3B in Kanga et al. (2005) (using the same data as Kanga et al. [2003]) also showed a spike immediately after treatment. To the extent these spikes exist, they are unlikely to be due to entomopathogenic fungi, because the fungi generally require >24 h to kill their host; indeed, Kanga et al. (2002) reported that the 95% confidence interval for the LT_{90} of *M. anisopliae* against varroa mites in laboratory bioassays was 5.48–7.43 d at 34 ± 1°C (similar to the temperature in the center of the hive). Few mites would therefore be expected to die from infection in <2 d. A more likely explanation is the physical effect of the treatment as a powder, rather than a pathogen, on the mites. Kanga et al. (2003, 2005) applied 46.8 and 93.6 g of *M. anisopliae* conidia per hive, or ≈4–8 times the quantity of powder applied in our study. Macedo et al. (2002) and Fakhimzadeh (2001) observed that many kinds of powders, such as powdered sugar, wheat flour, or pollen, cause mites to drop off bees. Because it is likely that the conidia formulation and the powder alone provoked an increase in mite fall unrelated to fungal infection, we excluded the data for the first sample after treatment from analyses for both experiments.

Percentage of infected mites was measured by placing sampled mites in conditions ideal for fungal sporulation. Hyphomycete fungi such as *M. anisopliae* and *B. bassiana* can grow readily on dying or freshly dead insects (Tanada and Kaya 1993), so the presence of a fungal colony on a mite cadaver does not necessarily mean that the fungus in question killed the mite. A mite, for example, with viable conidia on its cuticle may fall for other reasons, and in the 2–3 d between sampling occasions the conidia could germinate on the fresh cadaver, generating a false positive. Surface sterilization would remove some false positives but not necessarily all of them. Furthermore, some mites were recovered damaged, and surface sterilization of a damaged cadaver would likely have killed any infection, whether the fungus had killed the mite or not. However, a cadaver that failed to sporulate under the optimal growth conditions in the laboratory was considered unlikely to have died from the fungal infection. Thus, the percentage of infected mites as measured here should be considered upper bound estimates of the true percentage. The percentage of infected mites in both field experiments exceeded 60% for at least 2 wk after treatment (as with the mite fall data described above, the first sample after treatment in each experiment was excluded from analysis because of possible bias due to mites falling onto formulation that had filtered through the hive to the sticky boards). Untreated hives also showed increases in the percentage of infected mites. Kanga et al. (2003) qualitatively described infection of mites in untreated hives but did not provide time trend data.

The average cfu density per bee in treated hives immediately after treatment in the EBCL experiment, $\approx 1.1 \times 10^4$ cfu per bee, was less than that observed by Kanga et al. (2003), $\approx 6 \times 10^4$ to 2×10^5 cfu per bee, but higher than expected because the EBCL dose per hive was <1% of the "low-dust" dose of conidia used in Kanga et al. (2003). An average of $>10^3$ cfu per bee, associated with infection rates in excess of 80%, was accomplished with <0.4 g conidia, suggesting that the EBCL formulation was effective at dispersing conidia. However, although average cfu loads among bees from treated hives in this experiment declined to <20 cfu per bee in 3 wk, Kanga et al. (2003) observed average cfu densities in at least three treatments exceeding 100 cfu per bee after 6 wk. The dose–response curve presented here, itself a function of processes such as the percentage of infected mites increasing with cfu density, the infection incubation period, and the intrinsic and infection-induced mortality rate of phoretic and newly emerged mites, provides a first step to quantify the interaction between the microbial control agent and mite population dynamics.

Declines in cfu density on treated bees were attributed to a "decline in conidial viability" by Kanga et al. (2003). However, conidium viability is measured as a ratio of germinating conidia to the total number of conidia; thus, absent data on the total number of viable and nonviable conidia, cannot be reliably estimated from cfu density. A decline in cfu density can be considered a function of several processes, of which two important ones are the change in average conidium density per bee and the change in conidium viability over time, hereafter referred to as "conidium longevity." Average conidium density per bee changes for many reasons, including conidia falling off or being cleaned off bees, and the replacement of treated worker bees by emerging untreated bees.

Conidium longevity is itself a function of factors such as temperature, relative humidity, UV radiation, and contact with a host (Hedgecock et al. 1995; Fargues et al. 1996; Moore et al. 1996; Hong et al. 1997,

1999, 2001, 2002), and these factors usually depend on the location of the conidium. Although the role of these factors in conidium longevity for the isolate used here has not been quantified, longevity can be roughly estimated from published data for other isolates. During these experiments, ambient temperatures ranged from 0 to 29°C. In general, the maximum temperature conidia would have experienced in these experiments would have varied from as high as the center of the hive, typically 33–36°C (Winston 1987, Southwick 1991) to as low as the ambient temperature, such as when bees were outside the hive. Simpson (1961) reported relative humidity in hives between 40 and 50%, with periods as high as 70% when bees were using evaporative cooling. The relationship of conidium longevity to temperature and moisture content is generally inverse (Hong et al. 1997, 2001). Assuming the most extreme conditions for the conidia: the center of the hive during high humidity, or ≈35°C and 70% RH, and using a distributed delay model of conidial longevity (Meikle et al. 2003), the half life of eight *B. bassiana* isolates described in Hong et al. (2001) was estimated to be between 4 and 13 d. At 35°C and 40% RH, the half-life would be expected to be between 43 and 135 d. Few bees would spend all of their time in high-temperature brood areas, and cooler temperatures would increase conidium half-life. Exposure to UV light causes a drastic decrease in conidial longevity but would only be a factor for foraging bees.

Due to the complex dynamics of the bee–mite–fungus system, which is mainly played out within each hive but also includes interactions among hives, the interpretation of results from such experiments is not straightforward. Time trends in percentage infected mites, mite fall and cfu density are all important for interpretation, because they provide clues to identify changes among hives. The time trend of percentage of infected mites clearly showed that treatment caused additional infection and occasionally mite fall. Kanga et al. (2002, 2003, 2005) did show increased mite fall immediately after treatment but did not show the time trend of percentage of infected mites to support the hypothesis that infection (and not, for example, the powder effect described by Macedo et al. [2002]) was the cause. Indeed, Fig. 3 in Kanga et al. (2002) and Fig. 5 in Kanga et al. (2003) leave out the time factor and suggest, by the use of simple linear regression, that the percentage of infected mites is constant up to 42 d after treatment despite the steep decline in cfu density on bees (fig. 1 in Kanga et al. [2003]).

In treating colonies with a known insect pathogen, the potential impact of the pathogen on colony health is second in importance only to the impact of the fungus on the varroa mites. The infection of bee larvae by *B. bassiana* 05002 reported by Meikle et al. (2006b) is cause for concern. A virulent fungus introduced into a beehive might conceivably destroy the brood. However, social insects have an advantage over asocial insects in that a colony has properties associated with its complexity that are not found in individuals. An example might be found with termites, another social insect. Although adaptations to resist pathogens are found at the level of the individual, in the form of physiological immunity and inhibitory secretions, they are also found at the colony level, in the form of behavior such as allogrooming and the isolation and removal of disease-killed termites (Rosengaus et al. 1998a,b; Rath 2000, Osbrink et al. 2001, Su et al. 2003). Meikle et al. (2005) treated Formosan subterranean termites, *Coptotermes formosanus* Shiraki, with entomopathogenic fungi *Paecilomyces fumosoroseus* (Wize) Brown and Smith and then kept them either singly or as groups. They found that although individuals kept alone had a low median survivorship compared with controls, treated termites kept as groups had a higher survivorship, and treated individuals placed with untreated nestmates had survivorships higher still and similar to untreated nestmates. Although bee ecology and behavior are very different from those of termites, there may be similar protections against pathogens.

We did not try here to detect infected adult bees. Given the cfu density per bee, how quickly fungi can attack a fresh cadaver, and the unknown length of time between a bee's death and its being ejected from the hive, the presence of fungal growth on cadavers collected outside the hive was not seen as a reliable indicator of impact of fungus on the colony. Bees die for various reasons every day. A hive with 1.5 kg of adult bees (≈10,000 workers), assuming an average life span of 40 d (Winston 1992) and a stable population size, would lose an average 250 workers per day. Our objective here was to focus on the population of living bees and brood. We had too few replicate colonies per treatment for a definitive analysis of the impact of fungi on hive health, but even a qualitative examination of the results might shed some light on the potential for pathogens as a varroa management option. The mass of adult bees changed over time in hives treated with either conidia formulation or powder alone, but change is expected in a dynamic system. The amount of sealed brood of one colony treated with powder alone dropped to zero in the week after treatment, but this was related to a preexisting problem with the queen for that colony and in any case the hives treated with powder alone had low infection rates. No infection was observed in the frame photos and none could be detected in the data on the amount of sealed brood.

No colonies died at EBCL but one of the four treated colonies in the Lattes experiment was lost. Kanga et al. (2003) reported that four of 24 hives treated with fungi "swarmed (or left the bee hive)" within days after treatment, compared with one of 12 untreated hives. Although the colony loss in the experiment of Kanga et al. (2003) cannot be attributed to treatment (Fisher exact test: $P_{\text{one-tailed}} = 0.45$), a close examination of the impact of entomopathogenic fungi on hive health in general clearly needs to be conducted.

We have shown that the application of the conidia of fungi occurring naturally in beehives is associated with greatly increased percentages of infected mites and with significantly increased daily mite fall. These results are a promising step toward developing new

control strategies for varroa. The dosages used here were selected as being sufficient, based on our past experience with entomopathogenic fungi, without being excessive, because conidia are generally expensive, but studies on dose response are needed. Considering the dynamics of mite fall, percentage of infection, and cfu density per bee observed here, future work also should explore the effects of a second treatment per hive, probably after 1 or 2 wk when cfu density per bee had declined.

Acknowledgments

We thank K. Kristensen for statistical advice, and F. Derouané, W. A. Jones, B. G. Rector, and two anonymous reviewers for support and help in improving the manuscript.

References Cited

Chandler, D., K. D. Sunderland, B. V. Ball, and G. Davidson. 2001. Prospective biological control agents for *Varroa destructor n. sp., an important pest of the European honey bee, Apis mellifera.* Biocontrol Sci. Technol. 11: 429–448.

Davidson, G., K. Phelps, K. D. Sunderland, J. K. Pell, B. V. Ball, K. E. Shaw, and D. Chandler. 2003. Study of temperature-growth interactions of entomopathogenic fungi with potential for control of *Varroa destructor* (Acari: Mesostigmata) using a nonlinear model of poikilotherm development. J. Appl. Microbiol. 94: 816–825.

De Jong, D., R. A. Morse, and G. C. Eickwort. 1982. Mite pests of honey bees. Annu. Rev. Entomol. 27: 229–252.

Elzen, P. J., F. A. Eischen, J. B. Baxter, J. Pettis, G. W. Elzen, and W. T. Wilson. 1998. Tau-fluvalinate resistance in *Varroa jacobsoni* from several geographic locations. Am. Bee J. 138: 674–676.

Elzen, P. J., and D. Westervelt. 2002. Detection of coumaphos resistance in *Varroa destructor* in Florida. Am. Bee J. 142: 291–292.

Fakhimzadeh, K. 2001. Effectiveness of confectioner sugar dusting to knock down *Varroa destructor* from adult honey bees in laboratory trials. Apidologie 32: 139–148.

Fargues, J., M. S. Goettel, N. Smits, A. Ouedraogo, C. Vidal, L. A. Lacey, C. J. Lomer, and M. Rougier. 1996. Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic Hyphomycetes. Mycopathologia 135: 171–181.

Goettel, M. S., and G. D. Inglis. 1997. Fungi: hyphomycetes, pp. 213–249. *In* L Lacey [ed.] Manual of techniques in insect pathology. Academic, San Diego, CA.

Hedgecock, S. D. Moore, P. M. Higgins, and C. Prior. 1995. Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. Biocontrol Sci. Technol. 5: 371–377.

Hong, T. D., R. H. Ellis, J. Gunn, and D. Moore. 2002. Relative humidity, temperature, and the equilibrium moisture content of conidia of *Beauveria bassiana* (Balsamo) Vuillemin: a quantitative approach. J. Stored Prod. Res. 38: 33–41.

Hong, T. D., R. H. Ellis, and D. Moore. 1997. Development of a model to predict the effect of temperature and moisture on fungal longevity. Ann. Bot. 79: 121–128.

Hong, T. D., J. Gunn, R. H. Ellis, N. E. Jenkins, and D. Moore. 2001. The effect of storage environment on the longevity of conidia of *Beauveria bassiana.* Mycol. Res. 105: 597–602.

Hong, T. D., N. E. Jenkins, and R. H. Ellis. 1999. Fluctuating temperature and the longevity of conidia of *Metarhizium flavoviride* in storage. Biocontrol Sci. Technol. 9: 165–176.

Kanga, L.H.B., R. R. James, and D. G. Boucias. 2002. *Hirsutella thompsonii* and *Metarhizium anisopliae* as potential microbial control agents of *Varroa destructor,* a honey bee parasite. J. Invertebr. Pathol. 81: 175–184.

Kanga, L.H.B., W. A. Jones, and R. R. James. 2003. Field trials using the fungal pathogen, *Metarhizium anisopliae* (Deuteromycetes: Hyphomycetes) to control the ectoparasitic mite, *Varroa destructor* (Acari: Varroidae) in honey bee, *Apis mellifera* (Hymenoptera: Apidae) colonies. J. Econ. Entomol. 96: 1091–1099.

Kanga, L.H.B., W. A. Jones, and R. R. James. 2005. Enlisting fungi to protect the honey bee. Biologist 52: 88–94.

Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Macedo, P. A., J. Wu, and M. D. Ellis. 2002. Using inert dusts to detect and assess varroa infestations in honey bee colonies. J. Apic. Res. 41: 3–7.

Marascuilo, L. A., and J. R. Levin. 1983. Multivariate statistics in the social sciences: a researcher's guide. Brooks/Cole Publishing Company, Monterey, CA.

Martin, S. J. 2001. The role of *Varroa* and viral pathogens in the collapse of honey bee colonies: a modelling approach. J. Appl. Ecol. 38: 1082–1093.

Meikle, W. G., N. Holst, G. Mercadier, F. Derouané, and R. R. James. 2006a. Using balances linked to dataloggers to monitor honeybee colonies. J. Apic. Res. 45: 39–41.

Meikle, W. G., G. Mercadier, V. Girod, F. Derouané, and W. A. Jones. 2006b. Evaluation of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) strains isolated from varroa mites in southern France. J. Apic. Res. 45: 219–220.

Meikle, W. G., S. Jaronski, G. Mercadier, and P. C. Quimby. 2003. A distributed delay routine-based simulation model of *Beauveria bassiana* conidial stability in response to environmental stressors. Biol. Control 48: 561–578.

Meikle, W. G., G. Mercadier, R. B. Rosengaus, A. A. Kirk, F. Derouané, and P. C. Quimby. 2005. Evaluation of an entomopathogenic fungus, *Paecilomyces fumosoroseus* (Wize) Brown and Smith (Deuteromycota: Hyphomycetes) obtained from Formosan subterranean termites (Isoptera: Rhinotermitidae). J. Appl. Entomol. 129: 315–322.

Milani, N. 1999. The resistance of *Varroa jacobsoni* Oud. to acaricides. Apidologie 30: 229–234.

Moore, D., P. M. Higgins, and C. J. Lomer. 1996. Effects of simulated and natural sunlight on the germination of conidia of *Metarhizium flavoviride* Gams and Rozsypal and interactions with temperature. Biocontrol Sci. Technol. 6: 63–76.

Osbrink, W.L.A., K. S. Williams, W. J. Connick, Jr., M. S. Wright, and A. R. Lax. 2001. Virulence of bacteria associated with the Formosan subterranean termite (Isoptera: Rhinotermitidae) in New Orleans, LA. Environ. Entomol. 30: 443–448.

Rath, A. C. 2000. The use of entomopathogenic fungi for control of termites. Biocontrol Sci. Technol. 10: 563–581.

Rinderer, T. E., L. I. de Guzman, G. T. Delatte, J. A. Stelzer, V. A. Lancaster, V. Kuznetsov, L. Beaman, R. Watts, and J. W. Harris. 2001. Resistance to the parasitic mite *Varroa destructor* in honey bees from far-eastern Russia. Apidologie 32: 381–394.

Rosengaus, R. B., M. R. Guldin, and J.F.A. Traniello. 1998a. Inhibitory effect of termite fecal pellets on fungal spore germination. J. Chem. Ecol. 24: 1697–1706.

Rosengaus, R. B., A. B. Maxmen, L. E. Coates, and J.F.A. Traniello. 1998b. Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis augusticollis* (Isoptera: Termopsidae). Behav. Ecol. Sociobiol. 44: 125–134.

Shaw, K. E., G. Davidson, S. J. Clark, B. V. Ball, J. K. Pell, D. Chandler, and K. D. Sunderland. 2002. Laboratory bioassays to assess the pathogenicity of mitosporic fungi to *Varroa destructor* (Acari: Mesostigmata), an ectoparasitic mite of the honeybee, *Apis mellifera.* Biol. Control 24: 266–276.

Simpson, J. 1961. Nest climate regulation in honeybees. Science (Wash., D.C.) 133: 1327–1333.

Southwick, E. E. 1991. The colony as a thermoregulating superorganisms, pp. 28–47. *In* L. J. Goodman and R. C. Fisher [eds.], The behavior and physiology of bees. CAB International, Ascot, United Kingdom.

Su, N.-Y., Z. Hillis-Starr, P. M. Ban, R. H. Scheffrahn. 2003. Protecting historic properties from subterranean termites: a case study with Fort Christiansvaern, Christiansted National Historic Site, United States Virgin Islands. Am. Entomol. 49: 20–32.

Tanada, Y., and H. K. Kaya. 1993. Insect pathology. Academic, San Diego, CA.

Winston, M. L. 1987. The biology of the honey bee. Harvard Univ. Press, Cambridge, MA.

Winston, M. L. 1992. The honey bee colony: life history, pp. 197–234. *In* J. M. Graham [ed.] The hive and the honey bee. Dadant & Sons, Hamilton, IL.

Received 21 July 2006; accepted 30 October 2006.